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IN VITRO PHENOTYPIC ANTIBIOTIC SUSCEPTIBILITY PROFILES OF FOOD INDICATOR BACTERIA ISOLATED FROM HOME-MADE ORAL REHYDRATION SOLUTIONS IN NIGERIA

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ABSTRACT

One thousand and ten bacterial isolates from ORS constituents characterised as *Bacillus cereus* var. *mycoides*, *Bacillus subtilis*, *Citrobacter* sp., *Clostridium perfringes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *P. vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar* Typhi, *Salmonella enterica serovar* Typhimurium, *Shigella dysentariae*, *Staphylococcus aureus* and *Vibrio cholerae* were screened for their *in vitro* antibiotic susceptibility profiles using the agar discs and agar well-diffusion methods. The Gram-negative bacteria from granulated sugar samples had 7.69% phenotypic resistance profiles while the Gram-negative bacteria from table salt samples had between 13.3% and 20.0% resistance profiles. The resistance profiles of Gram-positive bacteria from granulated sugar samples was between 8.0% and 19.0% while the Gram-positive bacteria from table salt samples had between 11.0% and 27.9 % resistance profiles towards the test antibiotic (discs). The bacterial isolates from granulated sugar exhibited resistance of between 36.4% in ampicillin + cloxacillin and 64.9% in metronidazole. while the bacterial isolates from table salt gave an overall resistance of 41.0% - 64.7% towards the twenty-eight test oral paediatric antibiotic suspensions All the bacterial isolates from the table salt and granulated sugar samples displayed multiple resistance to the test paediatric antibiotics, except *Ps. aeruginosa* SA12, *Shigella dysenteriae* SA16C, SA16D, *E. aerogenes* SA18A, SA18AE and *E. coli* SA22A which recorded no (0.0%) resistance to all the test paediatric antibiotics.

KEYWORDS*: antibiotics,* **in vitro***, ORS, paediatric, resistance, susceptibility,*

INTRODUCTION

In the living conditions prevalent in the less developed world, characterised by a lack of potable water, sanitation, and refrigeration, the bacteria and other pathogens that cause diarrhoeal diseases are easily transmitted to young children by contaminated water, hands, and food. As a result, infants in less developed countries suffer on the average six to eight separate episodes of diarrhoeal disease per child (1). In an extremely conservative estimate, investigators calculated that there are at least 750 million to 1 billion episodes of diarrhoea and 4.6 million deaths each year due to diarrhoea in children less than 5 years of age in Africa, Asia (excluding the People's Republic of China), and Latin America (2).

Dehydration is the most important complication of infantile diarrhoea, and if untreated it can lead to death (3). Irrespective of the specific infectious agent causing diarrhoea, the treatment of diarrhoeal dehydration is the same, and involves the replacement of body water and electrolytes (salts in solution). A simple, efficacious, technologically appropriate alternative to intravenous dehydration has become available namely, oral dehydration therapy (ORT) using sugar/ electrolyte solutions. An application of ORT involves its use early in the

course of infant diarrhoea to prevent dehydration (4). It is not possible economically or logistically however to provide a packet of balanced sugar/electrolyte powder to treat every episode of diarrhoea in all young children in developing countries, for that reason, some observers have advocated the use of simple dehydration solutions of table salt and sugar, prepared and administered in the home (5). Several methods have been devised for the preparation of simple sugar/salt solutions that are safe and can be prepared in the home, while the ingredients used in oral dehydration solutions are widely available and easily transported (6)(7). The primary focus of this study therefore, is on the clinical significance of antibiotics as discs and oral paediatric suspensions on bacterial isolates obtained from home-made ORS constituents.

MATERIALS AND METHODS

Samples' collection: Five hundred granulated sugar and three hundred and seventy table salt samples obtained from the Federal capital territory, Abuja; five southwestern states- Lagos, Ogun, Oyo, Osun, Ekiti and Kogi state (a middle belt state) of Nigeria between February, 2001 and December, 2004 were microbially analysed in the laboratory to determine their microbial contents.

Isolation of the microbial flora of the samples: The overnight broth culture (1 ml) of each table salt and granulated sugar samples in alkaline peptone water (pH 8.6) was transferred into sterile plates by plating decimal dilutions of each sample in triplicates, and molten (45^oC) nutrient agar (NA; LAB M), thiosulphate citrate bile sucrose (TCBS; Oxoid) agar, pH 8.2; mannitol salt agar (MSA; LAB M), MacConkey agar (Oxoid), (LAB M) at pH 7.4, cystein lactose electrolyte deficient (CLED; LAB M) and Sabouraud dextrose agar (SDA; LAB M) were aseptically added to the plates and incubated between 24-48 hours at 35^oC for bacterial isolation and at 25^0C for fungal isolation (8). The population, in colony-forming units (CFU), and the characteristics of the colonies were recorded for each medium Purification and preservations of the isolates: Representatives of each different bacterial colony types were randomly picked from the primary plates of each sample and sub-cultured onto sterile plates by the streaking method. The isolates were then sub-cultured by repeated streaking to obtain pure cultures. All the bacterial isolates were kept at 4^0C in triplicates, on Brain Heart Infusion (BHI) agar slants as working and stock cultures.

Characterisation and identification of the isolates: Taxonomic studies were carried out on the purified isolates from the differently analysed samples on the basis of their cultural, morphological, biochemical and physiological characteristics, Tentative identification of the bacterial species was based on the conventional standard phenotypic taxonomic identification characteristics of the strains while the general key used for the identification was by reference to Kloos & Schleifer (9) and Bergey's Manual of Systematic Bacteriology (10).

Antibiotic susceptibility determination-

Agar disc diffusion method: Seeded Mueller-Hinton agar plates were left for about 15 minutes before aseptically placing the antibiotic discs [amoxicillin (25µg), augmentin (30µg), cotrimoxazole (25µg), nitrofurantoin (300µg), gentamicin (10µg), nalidixic acid (30µg) and tetracycline (30µg) for the Gram-negative bacteria; and ampicillin (10µg), chloramphenicol (30µg), cloxacillin (5µg), erythromycin (5µg), gentamicin (10µg), penicillin (15µg), streptomycin (10mg) and tetracycline (10µg)] on the agar surfaces and incubating the plates aerobically at 37^0 C for 18-24hr. Zones of inhibition and the diameter of the

zones were measured and recorded in millimeter diameter.

Agar well-diffusion method: Antibiotic susceptibility determination of various paediatric antibiotic suspensions (ampicillin/ampicillin-cloxacillin, cotrimoxazole, metronidazole, chloramphenicol, cephalexin and erythromycin) was carried out on the bacterial isolates using the modified agar disc and agar well-diffusion methods. Seeded Mueller-Hinton agar plates for bacterial isolates were prepared by transferring 500µl culture broth of each bacterium unto the agar plates followed by surface streaking of the entire agar surface with sterile wire loop. The seeded agar plates were then left for about 15 minutes before aseptically placing the antibiotic discs onto the agar surfaces and incubating the plates at 35^0C for 18-24 h. Zones of inhibition were measured and recorded in millimeter diameter according to the methods of NCCLS (11) and the modified method of Ogunshe (12) in which sterile semi-solid agar was added to the paediatric antibiotic suspensions to avoid spillage on the agar surface during the agar well-diffusion method.

RESULTS

One thousand and ten bacterial isolates characterised as *Bacillus cereus* var. *mycoides*, *Bacillus subtilis*, *Citrobacter* sp., *Clostridium perfringes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *P. vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar* Typhi, *Salmonella enterica serovar* Typhimurium, *Shigella dysentariae*, *Staphylococcus aureus* and *Vibrio cholerae* obtained from retailed table salt and granulated sugar samples were screened *in vitro* for their phenotypic antibiotic susceptibility/resistance profiles.

Apart from *Vibrio cholerae* SA12A which displayed 100.0% resistance to all the test antibiotics, a very high phenotypic susceptibility profiles to the test antibiotics were recorded among the Gram-negative bacteria from retailed table salt samples. However, out of the 8 test antibiotics (discs) assayed for in this study, antibiotic resistance of 7.69 % was displayed by all the Gram-negative bacteria isolated from retailed sugar samples towards all the test antibiotics while antibiotic resistance of 13.3 % was exhibited by the Gram-negative bacteria against augmentin, cotrimoxazole, nalidixic acid, nitrofurantoin, ofloxacillin, tetracycline and 20.0 % against amoxicillin and gentamicin (Table 1).

The antibiotic resistance recorded among the Gram-positive bacteria from retailed granulated sugar samples were gentamicin (8.0 %), chloramphenicol (10.0 %), erythromycin (13.6 %), streptomycin (15.4 %), ampicillin, cloxacillin, penicillin (16.7 %) and tetracycline (19.0 %). The Gram-positive bacteria from retailed table salt samples had resistance profiles of tetracycline (11.0 %), chloramphenicol (12.6 %), streptomycin (17.0 %), ampicillin (18.7 %), cloxacillin (20.7 %), erythromycin (23.2 %), penicillin (25.7 %) and gentamicin (27.9 %) respectively (Table 2).

The *in vitro* results of the antibiotic susceptibility patterns of the bacterial isolates from the table salt samples against the twenty-eight oral paediatric antibiotic suspensions gave resistance profiles of 36.4 % in ampicillin+ cloxacillin, 38.3 % in erythromycin + ethylsuccinate, chloramphenicol (40.2 %), cotrimoxazole (42.8 %), sulfamethoxazole + trimethoprim (42.9 %), cephalexin (44.4 %), erythromycin (49.5 %), ampicillin (51.9 %), amoxicillin (54.2 %) and 64.9% in metronidazole.. All the bacterial isolates displayed multiple resistance to the test antibiotics as shown in Table 2.

The *in vitro* antibiotic susceptibility patterns of the bacterial isolates from the granulated sugar samples exhibited mono and multi resistance to the twenty eight oral paediatric antibiotic suspensions (Tables 2 & 3).

The overall antibiotic resistance exhibited by the Gram-positive bacterial isolates from granulated sugar samples exhibited resistance profiles of 49.7 % in ampicillin, 50.6 % in cotrimoxazole, 52.1 % antibiotics. in cephalexin, 54.3 % in sulfamethoxazole + trimethoprim, 63.5 % in metronidazole and 64.7 % in chloramphenicol. All the bacterial isolates from the table salt samples exhibited mono and multiple antibiotic resistance (MAR) to all the test paediatric antibiotics, except *Ps. aeruginosa* SA12, *Shigella dysenteriae* SA16C, SA16D, *E. aerogenes* SA18A, SA18AE and *E. coli* SA22A which recorded 100.0 % susceptibility to all the test paediatric

Table 1: The antibiotic resistance profiles (antibiotic discs) of Gram-negative bacterial isolates from retailed granulated sugar and table salt samples

		Antibiotic resistance	
Antibiotic	Conc.	Sugar	Salt
Amoxicillin	25µg	7.69 %	20.0 %
Augmentin	30µg	7.69 %	13.3 %
Cotrimoxazole	25µg	7.69 %	13.3 %
Gentamicin	10µg	7.69 %	20.0 %
Nalidixic acid	30µg	7.69 %	13.3 %
Nitrofurantoin	30µg	7.69 %	13.3 %
Ofloxacillin		7.69 %	13.3 %
Tetracycline	30µg	7.69 %	13.3 %

Table 2: The antibiotic resistance profiles (antibiotic discs) of Gram- positive bacterial isolates from retailed granulated sugar and table salt samples

		Antibiotic resistance	
Antibiotic	Conc.	Sugar	Salt
Chloramphenicol 30µg	10.0 %	12.6 %	
Erythromycin	5µg	13.6 %	23.2 %
Gentamicin	10µg	8.0 %	27.9 %
Streptomycin	10mg	15.4 %	17.0 %
Tetracycline	10µg	19.0 %	11.0 %
Ampicillin	10µg	16.7 %	18.7 %
Cloxacillin	5µg	16.7 %	20.7 %
Penicillin	15µg	16.7 %	25.7 %

Table 3: The antibiotic resistance profiles (Oral paediatric suspensions) of Gram-negative bacterial isolates from retailed granulated sugar and table salt samples

Antibiotics	Antibiotic resistance	
	Sugar	Salt
Ampicillin + cloxacillin	36.4 %	32.8 %
Erythromycin + ethylsuccinate	38.3 %	41.3 %
Chloramphenicol	40.2 %	64.7 %
Cotrimoxazole	42.8 %	50.6 %
Sulfamethoxazole + trimethoprim	42.9 %	54.3 %
Cephalexin	44.4 %	52.1 %
Erythromycin	49.5 %	43.2 %
Ampicillin	51.9 %	49.7 %
Amoxicillin	54.2 %	41.0 %
Metronidazole	64.9 %	63.5 %

DISCUSSION

Fluid replacement therapy that is widely used in medicine in prevention or treatment of dehydration, or as an intravenous therapy to prevent hypovolemic shock, and this may be the reason for table salt being one of the compositions of home-made ORS in addition to sucrose sugar. The significance of ORS is to alleviate morbidity and mortality through fluid loss during gastroenteritis/diarrhoea (13), however, the finding of Ogunshe *et al*. (14) indicates that home-made ORS may serve as means of transmitting gastroenteritis/diarrhoeal and other infectious microbial agents due to the high recovery rates of the indicator bacteria from retailed table salt and granulated sugar samples, more especially from home-made ORS solutions. These bacteria have however, been previously implicated in clinical cases (15)(16)(17)(18).

Though the magnitude of the problem may vary from place to place, the problem of antibiotic resistance is probably amplified in tropical developing countries where infectious conditions account for a substantial percentage of hospital consultations. Several workers in the country and elsewhere have highlighted the problem of antibiotic resistance (19)(20). The bacterial pathogens in this study were assayed for their susceptibility to eight (µg/disc) commonly used antibiotics incorporated in multi-discs, and it was noted that the phenotypic antibiotic resistance profiles of the bacterial isolates used in this study were relatively low except among the Gram-positive bacterial isolates. The results obtained in this study indicated that the Gram-negative bacterial isolates were highly susceptible to the test antibiotics (discs), which is in conformity with the reports of some earlier workers such as Dax (21)(22), but contrary to the previous findings of Ryan *et al*. (23), Chopra *et al*. (24) and Hlavka *et al*. (25) who had all earlier reported a high resistance to same antibiotics by certain clinical bacterial isolates. The high prevalence of susceptibility of the bacterial isolates to nalidixic acid in this study may be attributable to similar finding which stated that nalidixic acid displays good activity against certain Gram-negative pathogens but that a wide variety of bacteria that are resistant to quinolones have been selected from laboratory strains or have been obtained from clinical isolates (22). It could therefore be inferred that the high level susceptibility to nalidixic acid by the bacterial isolates from the ORS constituents (table salt and sucrose sugar) in this study was because the pathogens were non-clinical isolates. Koneman *et al*. (26) reported that aminoglycoside antibiotics,

such as streptomycin and gentamicin, are bactericidal and tend to be most active against Gram-negative pathogens. This may account for the low resistance among the Gram-negative bacterial isolates but very high resistance observed among the gram-positive bacterial isolates in this study.

Examples of penicillins according to Dax (22) are ampicillin; amoxicillin etc., while amoxyillin with clavulanic acid is termed augmentin. The fact that a lower antibiotic resistance was recorded in ampicillin in this study however confirms the earlier findings of Neu (27) that amplicillin is the most recognized of the aminopenicillins and remains a valuable and widely prescribed chemotherapeutic agent as well as being markedly more active against a host of Gram-negative bacilli. Amoxicillin has been claimed by Dax (21) to be essentially comparable to ampicillin in terms of *in vitro* potency. The results obtained in this present study, in which low antibiotic resistance was displayed against amoxicillin by the Gram-positive and Gram-negative bacterial isolates confirms the earlier report of Dax (21).

Schwan & Ebetino (28) earlier reported that the nitrofurans such as nitrofurantoin are medium-spectrum antibacterial agents, which are potent against a variety of Gram-positive and Gram-negative bacteria, assuming that sufficient concentrations are achieved at the site of infection and that resistance is not a problem with the use of these agents. Similarly, Brooks *et al*. (29) supported the potency of gentamicin in a large percentage of bacterial isolates. Having recorded very low resistance to nitrofurantoin in this study supports the earlier findings of Schwan & Ebetino (28) and Brooks *et al*. (29), however, a very high resistance to gentamicin by the Gram-positive bacterial isolates in this study disagrees with the earlier findings of Schwan & Ebetino (28) and Brooks *et al*. (29).

Several antimicrobial agents have become available for use in newborns and children with suspected or proven bacterial infections, but the most commonly employed method for antibiotic susceptibility screening is usually the agar disc-diffusion method, using antibiotic discs. Clinically, infantile antibiotic prescriptions are generally made based on the antibiotic (discs) reports; meanwhile, paediatric antibiotic suspensions are usually administered on infants and children. No documented reports on the antibiotic susceptibility patterns of bacterial isolates using paediatric suspensions was obtained except for those of Ogunshe (12) and the results obtained in this study are in accordance with their earlier findings which indicated that infantile gastroenteritic and non-gastroenteritic bacterial isolates were more resistant to paediatric oral suspensions, especially amoxicillin, ampicillin, co-trimoxazole, chloramphenicol, sulfamethazole + trimethroprim and metronidazole. In this study, the *in vitro* results of the antibiotic susceptibility of the bacterial isolates to various paediatric oral suspensions gave a relatively higher percentage resistance than those of antibiotic discs. These findings indicate that the bacterial isolates were more resistant to the paediatric oral suspensions. Recording as high as 49.5% - 64.9% resistance in paediatric antibiotics such as amoxicillin, ampicillin, chloramphenicol, co-trimoxazole, erythromycin, metronidazole and sulfamethazole+trimethroprim shows the health implications that these commonly prescribed and consumed antibiotics may pose in paediatric infectious conditions, especially since it has already been reported that antibiotic resistance is a world-wide problem that is also prevalent in Nigeria (30)(31)(32). Although percentage resistance of other classes of paediatric antibiotics

such as ampicillin and erythromycin+ethylsuccinate suspensions were relatively lower, the percentage resistance is still higher than expected in paediatric chemotherapy having found widespread use particularly as children suspensions.

All the isolated bacteria species isolated from retailed table salts and granulated sugar samples in this study exhibited mono and multi resistance to all the test antibiotic discs and paediatric antibiotic suspensions which indicate the unwholesomeness and clinical implication of the samples especially as constituents of ORS in infantile therapy. This study has shown a worsening trend in the antibiotic resistance profiles of the bacterial isolates. From the overall results of the antibiotic resistance profiles obtained in this study, it is therefore an established fact that the onset of drug resistance threatens virtually all classes of antibacterial agents as well as confirming the predictions of a worsening antibiotic resistance situation especially in paediatric chemotherapy which has become an accepted medical practice. The emergence of antimicrobial-resistant bacterial pathogens has become a major public health concern thus; the use of antimicrobials in any area including disease treatment can potentially lead to widespread dissemination of antimicrobial-resistant bacteria **(33)([34)(35)(36)(37)(38), more especially if such antimicrobial-resistant bacteria are from food sources such as granulated sugar and table salt samples**

All the bacteria species isolated from the table salt samples were mono- or multi-resistant to the test antibiotics and the danger in this phenomenon is that the multi-resistance determinants can be transferred to new bacterial hosts. The situation is made more difficult in developing countries such as Nigeria where antimicrobial drugs are readily available to consumers across the counter with or without prescriptions from medical practitioners. Such a practice can lead to misuse of the antimicrobial drugs with the associated high prevalence of drug resistance among the implicated bacterial isolates.

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